INDICATIONS REGARDING THE SOURCE OF COMBINED NITROGEN FOR ULVA LACTUCA

G. L. FOSTER

Teaching Fellow in the Henry Shaw School of Botany of Washington University

Introduction

Very little attention has been given the question of the sources of nitrogen for marine algæ. Nevertheless, the question is an interesting one both physiologically and ecologically, because of the extremely small amount of nitrogen supposed to be present in sea-water, and because of the very noticeable change in the type of algal flora when the nitrogen content of the environment is increased, as by the presence of sewage. The literature bearing on the subject is practically limited to a debate between a few authors as to the amount and form of nitrogen in sea-water, and the way in which the supply is maintained. This dispute involves some questions of fundamental importance for marine biology; consequently, a brief statement of the different views is pertinent.

Natterer (13) reports that careful analyses of water from the high seas show scarcely a trace of nitrates. Nitrites are somewhat more abundant, but not sufficiently so to admit of quantitative determination. Ammonium compounds, on the other hand, according to Thoulet, are present in sufficient amount to be quantitatively determined, and vary from .13 to .34 mg. per liter (.013–.034 per cent) according to the locality. Reinke (15) considers these amounts of nitrogen reported to be insufficient for the production of the enormous amount of living material in the sea, especially when the activity of nitrifying and denitrifying bacteria is taken into account. He considers as of prime importance in this question the nitrogen-fixing bacteria which have been demonstrated in sea-water by Benecke and Keutner (4), and others. Reinke found *Azotobacter* embedded in the gelatinous material on the surface of *Laminaria* fronds and argues for a symbiotic relation between the algæ and bacteria.

Brandt (7), however, attaches little or no importance to Reinke's view, and maintains that the nitrogen content of sea-water is determined by a balance between the activity of denitrifying bacteria, on the one hand, and the great amount of nitrogenous material carried to the sea by the rivers, on the other. Brandt (5, 6) considers that the nitrogen content of sea-water is at a "minimum" and is the limiting factor in the production of marine organisms. Considering especially the plankton life, he finds that the amount of plankton is proportional to the nitrogen content of the water, and correlates the comparative poverty of tropical seas in plankton life with the relatively greater activity of denitrifying bacteria in the warmer waters.

More recently Pütter (14) has reported that analyses of the water from the Gulf of Naples give per liter .18 mg. of nitrogen in nitrates and nitrites, and .56 mg. in ammoniacal nitrogen. Furthermore, he claims that these figures represent less than half the total combined nitrogen actually present in sea-water. In his opinion there is no need for considering the nitrogen content to be at a "minimum" since it is present in greater concentration than the carbon dioxide. It is impossible to say which of these views is the correct one, and further work in this field is much needed.

The above named authors incidentally assume that nitrogen is available for the algæ only in the form of nitrates or ammonium salts. This is entirely an a priori assumption, as no data are offered in support of such a view. On the contrary, it seems more likely that the algæ can use many organic nitrogen compounds. This would seem probable in the light of recent work which has been done on the fresh-water algæ.

In regard to the nutrition of the fresh-water forms we have departed far from the old idea that green plants are strictly autotrophic. Thus, by the work of Beyerinck (3), Charpentier (8), Chick (9), Artari (1, 2), and others, it has been established that many of the fresh-water algæ have, with respect to nitrogen, distinct saprophytic tendencies,—preferring organic to inorganic nitrogen. Artari, especially, has shown that several algæ (*Chlamydomonas*, *Stichococcus*, *Chlorella*, *Scenedesmus*, and others) can grow and retain the chlorophyll under completely saprophytic conditions, as in solutions containing amino acids and glucose in the absence of light and carbon dioxide. In all these cases, however, growth is more rapid under so-called mixotrophic conditions, i. e., with both organic nitrogen and carbon present in addition to sunlight and carbon dioxide. Artari takes up the question of the relative value of different nitrogenous compounds, and shows that they vary greatly with different algæ,—some preferring peptones, others, amino acids and ammonium salts, and a few, nitrates. On the whole, the majority of forms investigated grow best in the presence of amino nitrogen. Many algæ, especially of this last class, are often found in water polluted with sewage or decaying organic matter.

Among the marine algæ, there is a more or less definite flora characteristic of sewage-polluted waters. Most conspicuous among the plants of this group are the species of *Ulva.* Letts and Richards (11), in their reports on sewage in British harbors, state that *Ulva latissima* grows in excessive quantities in polluted waters, and they find that the nitrogen content of this seaweed varies with the degree of pollution of the water. Cultural experiments conducted by Letts and Richards showed that *Ulva latissima* grows more rapidly in a mixture of sewage and sea-water than in pure sea-water alone.

Experimental

Preliminary experiments were made at the Woods Hole Laboratory to determine the sources of available nitrogen for *Ulva lactuca.* The algal material used in the experiments was collected at the mouth of an inlet where the water was at all times highly polluted with sewage. The cultures were maintained in the laboratory in glass tumblers containing 150 cc. of solution. When brought in, the fronds of *Ulva* were well rinsed in clean sea-water and cut into strips exactly 3 cm. in length and about 2 cm. wide. Three such strips were placed in each vessel, and the cultures kept at a temperature of 21°C. by placing the vessels in a tray of running water. In each case the solution was renewed at the end of 5 days. After 10 days the strips were again measured and the increase in length recorded.

Two main types of nutrient solution were used,—one (solution A) being natural sea-water, the other (solution B) being an artificial sea-water minus nitrogen. These stock sea-waters were made double strength and subsequently diluted by the addition of distilled water and the stock solution of the nitrogenous compounds to be tested. The following nitrogen compounds were used in the experiments: ammonium nitrate, urea, acetamid, sodium asparaginate, acetanilid, and dimethylanilin. Parallel experiments were run, adding these compounds to solution A and solution B.

Preliminary tests roughly determined the maximum non-toxic concentrations of these compounds when added to sea-water to be:

Ammonium nitrate............	0.011 gram molecular
Urea..........................	0.010 gram molecular
Acetamid.....................	0.250 gram molecular
Asparagin....................	0.080 gram molecular

The table presents the results of the experiments. All figures for concentrations represent fractions of gram molecules per liter, except in the case of dimethylanilin. Here the solubility was not known and the figures represent fractions of a saturated solution in distilled water at 20°C. In the column headed "growth" is recorded the increase in length in millimeters of the strips of *Ulva* after 10 days in the solution. In each case the figures for growth represent the average of three or more cultures. Checks show the growth in solutions A and B with no additional nitrogen.

It is apparent from the following table that, under the conditions of the experiment, ammonium nitrate and urea are considerably better nutrients for *Ulva* than the other compounds used. These two cause a marked increase in growth over that of the controls, in both the artificial and natural sea-waters. The nutritive value of these compounds was also indicated by the healthy appearance of the cultures. The algæ were of a deep green color, very turgid, and considerably curled by rapid growth. Judging from the growth and general appearance of the cultures, there is little choice between the nutrient values of ammonium nitrate and urea.

COMPARATIVE TABLE SHOWING GROWTH OF STRIPS OF ULVA IN VARIOUS NITROGEN-CONTAINING SOLUTIONS

Ammonium nitrate			Urea			Acetamid		
Conc.	Growth		Conc.	Growth		Conc.	Growth	
	Sol.* A	Sol.* B		Sol.* A	Sol.* B		Sol.* A	Sol.* B
Check	0.8	0.3	Check	0.8	0.3	Check	0.8	0.3
0.00005	1.0	0.5	0.0005	1.4	0.5	0.001	0.8	0.4
0.0001	1.4	1.5	0.001	1.6	0.6	0.005	1.0	0.4
0.0005	1.9	2.0	0.005	1.6	1.4	0.01	0.7	0.5
0.001	1.4	1.2	0.01	0.9	1.3	0.10	1.0	1.0

Sodium asparaginate			Acetanilid			Dimethylanilin		
Conc.	Growth		Conc.	Growth		Conc.†	Growth	
	Sol.* A	Sol.* B		Sol.* A	Sol.* B		Sol.* A	Sol.* B
Check	0.8	0.3	Check	0.8	0.3	Check	0.8	0.3
0.002	0.7	0.6	0.0005	0.6	0.5	0.002	0.3	0.4
0.01	0.9	0.2	0.0025	0.0	0.0	0.02	0.0	0.5
0.05	0.7	0.0	0.0125	0.0	0.0	0.10	0.0	0.0

* Sol. A = natural sea-water; sol. B = artificial sea-water.

† Conc. under dimethylanilin represents fractions of a saturated solution in distilled water at 20°C.

Acetamid has a somewhat lower nutrient value than ammonium nitrate or urea, but still it causes a greater growth than do the control solutions to which no foreign nitrogen was added. The alga in acetamid solutions appeared normal in every way. The results with the sodium asparaginate were rather unexpected. This compound is well known to be a good nutrient for many fungi and fresh-water algæ. For *Ulva,* on the other hand, sodium asparaginate appears to have no appreciable nutrient value. In no case did it cause any notable increase in growth, although the algal material appeared perfectly normal.

Acetanilid and dimethylanilin are in a separate class,—being decidedly toxic at all the concentrations used. At the lowest concentrations there was slight growth at first, but in ten days all cultures were dead and discolored. The results with these last two compounds are comparable to those obtained with similar substances by Czapek (10) and by Lutz (12) working on fungi and fresh-water algæ. They found that compounds having the nitrogen attached directly to a benzene nucleus are toxic.

Pure culture methods were not attempted on account of the brief time available for this work, and the question of the possible interaction of ammonifying bacteria is therefore pertinent. However, the rapid augmentation of growth upon the addition of the amido compounds, and the comparative absence of bacteria both suggest a direct absorption of these substances. Moreover, since rapid growth of the alga occurs in concentrations of the amido compounds considerably greater than the toxic limit for ammonium salts, and since, further, no evidence of toxicity of fairly strong solutions of urea and acetamid developed during the interval of these experiments, no support is given to the thought that ammonification may be an important factor. However, in further continuation of this work it is proposed to control this possibility by quantitative tests.

It seems probable from the facts brought out here, as well as from the work of Letts and Richards, that *Ulva* is not limited to an inorganic nitrogen supply, since growth occurs with urea or acetamid as the sole source of nitrogen, and, as Letts and Richards have shown, that it grows more rapidly in sewage-polluted water than in pure sea-water. Undoubtedly, further experiments would show that other organic compounds can supply available nitrogen for *Ulva.*

The results also indicate that for *Ulva*, at least, the amount of available nitrogen in the water is the limiting factor in growth. This is shown by the fact that growth is more rapid in sea-water containing additional nitrogen (ammonium nitrate, or urea) than in pure sea-water. The above mentioned results of Letts and Richards also point to the same conclusion, as does the abundant growth of *Ulva* in nature in waters polluted with sewage.

In conclusion, the writer is pleased to express his thanks for the generous assistance given during this study by Prof. B. M. Duggar, under whose direction the work was carried out while occupying a research table maintained by Dartmouth College at the Marine Biological Laboratory, Woods Hole, Massachusetts.

Graduate Laboratory, Missouri Botanical Garden.

Literature Cited

1. Artari, A. Zur Frage der physiologischen Rassen einiger grünen Algen. Ber. d. deut. bot. Ges. **20**: 172–75. 1902.
2. ———, Zur Physiologie der Chlamydomonaden. Jahrb. f. wiss. Bot. **52**: 410–66. 1913.
3. Beyerinck, M. W. Culturversuche mit Zoochlorellen, Lichenengonidien und anderen niederen Algen. Bot. Zeit. **48**: 725–54, 757–67, 781–85. 1890.
4. Benecke, W., und Keutner, J. Über stickstoffbindende Bakterien aus der Ostsee. Ber. d. deut. bot. Ges. **21**: 333–46. 1903.
5. Brandt, K. Ueber den Stoffwechsel im Meere. Wiss. Meeresunters. N.F. Abt. Kiel **4**: 215–30. 1899.
6. ———, Ueber den Stoffwechsel im Meere. 2. Abhandl. Ibid. **6**: 25–79. 1902.
7. ———, Ueber die Bedeutung der Stickstoffverbindung für die Produktion im Meere. Beih. bot. Centralbl. **16**: 383–402. 1904.
8. Charpentier, P. G. Alimentation azotée d'une algue, le Cystococcus humicola. Ann. Inst. Pasteur **17**: 321–34, 369–420. 1903.
9. Chick, H. A study of a unicellular green alga occurring in polluted water, with special reference to its nitrogenous metabolism. Proc. Roy. Soc. **71**: 458–77. 1903.
10. Czapek, Fr. Zur Kenntniss der Stickstoffversorgung und Eiweissstoffwechsel bei Aspergillus niger. Ber. d. deut. bot. Ges. **19**: 130–39. 1902.
11. Letts, E. A., and Richards, E. H. On green sea weeds (especially Ulva latissima) in relation to the pollution of the waters in which they occur. Seventh Report of the Roy. Comm. on Sewage Disposal. Appendix 3: 72–100. 1911.
12. Lutz, L. Sur l'action exercée sur les végétaux par les composés azotées organiques a noyau benzenique. Compt. rend. Cong. Soc. Sav. Paris **1903**: 65–69.
13. Natterer, K. Chemische Untersuchungen von der Expedition S. M. Schiff "Pola." Denkschr. k. Akad. Wiss., Wien, math.-naturw. Kl. **65**: 445. 1898.
14. Pütter, A. Der Stoffhaushalt des Meeres. Zeitschr. f. allgem. Physiol. **7**: 321–68. 1907.
15. Reinke, J. Die zur Ernährung der Meeresorganismen disponiblen Quellen an Stickstoff. Ber. d. deut. bot. Ges. **21**: 371–80. 1903.